RADICAL CAUSES OF CANCER

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Free radicals are ubiquitous in our body and are generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganisms. Because free radicals can also inflict cellular damage, several defences have evolved both to protect our cells from radicals — such as antioxidant scavengers and enzymes — and to repair DNA damage. Understanding the association between chronic inflammation and cancer provides insights into the molecular mechanisms involved. In particular, we highlight the interaction between nitric oxide and p53 as a crucial pathway in inflammatory-mediated carcinogenesis.

CYTOKINE
A soluble protein that is produced and released by individual cells that transmit distinct messages of activation, inhibition, chemoattraction or apoptosis to other cells. This interaction triggers effector mechanisms within the responding cell. Key pro-inflammatory cytokines include IL-1β, TNF-α and IFN-γ.

FREE RADICAL
An atom or a group of atoms that has an unpaired electron. These are highly reactive to biological molecules and will damage them.

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The infectious and non-infectious generation of chronic injury and irritation initiates an inflammatory response[1,2]. 'Go' signals[2] — such as bioactive peptides from neurons, CYTOKINES or receptor molecules that sense microbes — lead to the recruitment of mast cells and leukocytes to the damage site. A subsequent 'respiratory burst' — an increased uptake of oxygen that leads to the release of FREE RADICALS from leukocytes, including activated macrophages — can damage otherwise healthy neighbouring epithelial and stromal cells. This process can drive carcinogenesis by altering targets and pathways that are crucial to normal tissue homeostasis (reviewed in REFS 1,3). It has been estimated that chronic infection and associated inflammation contribute to about one in four of all cancer cases worldwide[1,4].

Targets of free radicals in inflammation include DNA, proteins, RNA and lipids (FIG. 1). Mutations in cancer-related genes or post-translational modifications of proteins by NITRATION, NITROSATION, phosphorylation, acetylation or polyADP-ribosylation — by free radicals or LIPID PEROXIDATION byproducts, such as the reactive aldehydes MALONDIALDEHYDE and 4-hydroxy-nonenal — are some of the key events that can increase the cancer risk. In addition, free radicals can modulate cell growth and tumour promotion by activating signal-transduction pathways, which results in the transcriptional induction of proto-oncogenes, including c-FOS, c-JUN and c-MYC[5], that are involved in stimulating growth. Mechanistic studies have shown that protein phosphorylation and polyADP-ribosylation of chromosomal proteins are involved in the transcriptional induction of c-FOS by oxidants[6] and that a pro-oxidant state can promote neoplastic growth[7].

TABLE 1 summarizes examples of oxyradical overload diseases. These develop from conditions of chronic inflammation and can have an aetiology that is primarily inherited or acquired through viral, bacterial and parasitic infection, or acquired through chemical induction. Cancer proneness is frequently a pathological consequence of extensive and sustained free-radical stress-related damage in these diseases. However, the exact mechanisms still require further elucidation. What are the roles of free radicals in cancer proneness in chronic inflammatory — 'oxyradical overload' — diseases? What are the targets of these free radicals *in vitro* and *in vivo*? And, because the tumour suppressor p53 is a crucial target for alteration, how is this molecule involved in the genesis of cancer during chronic inflammation?

Free radicals damage DNA and modify proteins

The hypothesis that free radicals have a role in carcinogenesis comes from *in vitro* studies describing their role in DNA damage, and protein structural and functional modifications. One of the earliest convincing studies showing that free radicals damage DNA came from the observation that hydrogen peroxide (H_2O_2), in the presence of a perioxidation activator, $Fe_2(SO_4)_3$, induced chromosome fragmentation[8]. Since then, many others have shown an association of other free radicals with DNA damage and protein modifications[9–13].

NITRATION
Addition of an equivalent of $NO_2^{\bullet}$ in a free-radical mechanism, often resulting in the formation of 3-nitrotyrosine. This modification can affect the function of certain proteins and is involved in disease pathology, including cancer.

NITROSATION
Addition of an equivalent of NO^+ (nitrosonium ion or nitrosyl cation) to an amine, thiol or hydroxyaromatic group.

LIPID PEROXIDATION
Auto-oxidation of lipids that are exposed to oxygen.

Summary

- Chronic inflammation deregulates cellular homeostasis and can drive carcinogenesis.
- Free radicals and aldehydes — produced during chronic inflammation — can induce a number of alterations, including gene mutations and post-translational modifications of key cancer-related proteins. These alterations can lead to the disruption of cellular processes such as DNA repair, cell-cycle checkpoints and apoptosis.
- The ultimate effect of free radicals is complex and depends on their local concentration, the microenvironment and the genetic background of the individual.
- Nitric oxide and its derivatives damage DNA and modify protein structure and function but can also protect from cytotoxicity. These 'two faces' of nitric oxide highlight the need for further study before considering nitric oxide as a target for chemoprevention in high cancer risk, chronic inflammatory diseases.
- People with cancer-prone inflammatory diseases, such as ulcerative colitis, haemochromatosis and viral hepatitis, have alterations in cancer-related genes and proteins, which are associated with free-radical stress.
- Transgenic and knockout animal models support the role of free radicals in carcinogenesis.
- Prospective chemoprevention studies are needed to evaluate the use of antioxidants and inhibitors of pro-oxidant enzymes for the prevention of cancer in people with oxyradical overload diseases.

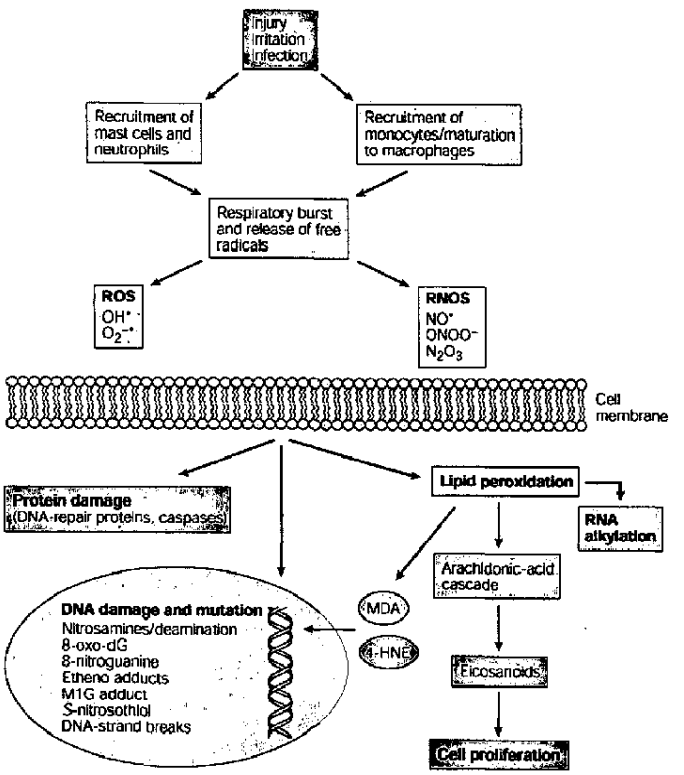

Figure 1 | **Impact of free radicals released at sites of inflammation on cellular molecules.** Inflammation begins with a reaction to an irritant or infection that is characterized by movement of fluid and white blood cells into extravascular tissue. This is followed by tissue repair and regeneration and involves cell proliferation. Associated with these processes are the release of free radicals, such as reactive oxide species (ROS) and nitrogen oxide reactive species (RNOS). This can activate a process called lipid peroxidation and the arachidonic-acid cascade, with the production of cell-proliferation-stimulating eicosanoids. Also, DNA-damaging agents, such as malondialdehyde (MDA) and 4-hydroxynonenol (4-HNE), are by-products of the arachidonic-acid cascade. The free radicals can also damage DNA and modify the structure and function of cancer-related proteins directly. OH•, hydroxyl radical; $O_2^{-\bullet}$, superoxide; NO•, nitric oxide; $ONOO^-$, peroxynitrite; N_2O_3, nitrous anhydride.

Due to the complex chemical and biological nature of free radicals, we are only now beginning to understand the key players involved in inflammatory-mediated carcinogenesis and their specific actions. In this review, we focus on the effects of free radicals on biomolecules in relation to carcinogenesis. The complex chemistry has been extensively reviewed elsewhere[3,14–16]. Highly reactive molecules to DNA *in vitro* include hyroxyl radicals (OH•), which cause oxidative DNA damage, and peroxynitrite ($ONOO^-$), which causes both oxidative damage and nitration of DNA bases. *In vivo*, the relevance of OH• to DNA damage, however, remains unclear because it has a very short half-life, and, therefore, has to be produced directly adjacent to DNA to induce damage[17]. By contrast, $ONOO^-$ can diffuse within cells[18], and so might be of particular concern during chronic inflammation. A less reactive molecule — such as nitric oxide (NO•) — has the ability to diffuse over several cell diameters[19] and might also be relevant. Reactive species that are derived from NO• are released from inflammatory cells and can act on neighbouring dividing epithelial cells, leading to somatic mutations in crucial cancer-causing genes.

Test-tube and cell-culture studies have shown that DNA is not the only molecule at risk. Proteins such as DNA-repair enzymes, those involved in signal transduction, apoptotic modulators and the p53 protein can be modified — both structurally and functionally — when exposed to free radicals[10]. Key post-translational modifications following free-radical exposure and their functional consequences have been reviewed elsewhere[20–22]. We[23] and others[24] have shown that, after exposure to NO• and its derivatives, p53 is post-translationally modified at residues that are crucial to its multiple functions. Others have shown that DNA-repair and signal-transduction molecules, such as DNA-protein kinases, are activated by exposure to NO• (REFS 25,26). As a modifier of proteins that are crucial to cell function, NO• can influence downstream events in carcinogenesis, including cell-cycle checkpoints[27], apoptosis[28] and DNA repair[29,30]. Elucidation of these influences has provided a better

Table 1 | **Examples of high cancer risk, oxyradical overload diseases**

Disease	Cancer	Risk*	References
Inherited			
Haemochromatosis	Liver	219	97
Crohn's disease	Colon	3	153
Ulcerative colitis	Colon	6	154
Acquired: viral			
Viral hepatitis B	Liver	88	155
Viral hepatitis C	Liver	30	155
Human papillomavirus infection	Cervix	16	156
Acquired: bacterial			
Helicobacter pylori infection	Gastric	10	157
Urinary bladder catheterization	Bladder	5–28	158
Prostatitis	Prostate	2	179
Acquired: parasitic			
Schistosoma hematobium	Bladder	2–14	134
Schistosoma japonicum	Colon	1.2–6.0	134
Acquired: chemical/physical			
Barrett's oesophagus	Oesophageal	50–100	159
Pancreatitis	Pancreatic	2–3	160

*Relative risk or odds ratio.

understanding of how a micro-environment of chronic inflammation can transform a normal cell into a cancer cell (FIGS 1 and 2).

The two faces of nitric oxide

Since its discovery, it has been difficult to identify the specific roles of NO• in carcinogenesis. This is because the effects of NO• are dependent on its concentration, its interaction with other free radicals, metal ions and proteins, and the cell type and the genetic background that it targets. It is fairly clear that NO• mediates angiogenesis[31–33]. However, NO• can both cause DNA damage and protect from cytotoxicity, it can inhibit and stimulate cell proliferation, and it can be both pro- and anti-apoptotic[34–46]. It is therefore important to be aware of the conditions used when interpreting results from cell-culture experiments[47]. Depending on the conditions used, DNA-damaging NO• byproducts — such as nitrosoperoxycarbonate, $ONOO^-$ and nitrogen dioxide — are often formed[48–51]. This complicated chemistry and the biological actions of NO• should also be considered when interpreting the results of animal studies[33,52].

Inflammation, nitric oxide and p53

Alterations in DNA occur after exposure to high levels of NO• and its derivatives[53–55]. p53 mediates the response to various stress signals, and exposure of NO• causes p53 accumulation and post-translational modifications[23] that inhibit cellular growth. This can lead to selective clonal expansion of p53-mutant cells following NO• exposure (FIG. 2). We have tested this hypothesis by genetically engineering human cancer cells *in vitro* to produce concentrations of NO• that are similar to those in human cancer. We observed an increase in the expression of the G1–S cell-cycle checkpoint protein, WAF1, in cells with wild-type p53, and also expression of the enzyme inducible NO synthase (iNOS), which catalyses the production of NO•, leading to reduced tumour growth and increased tumour necrosis as xenografts in athymic nude mice. By contrast, engineered cells with mutated p53 showed an accelerated tumour growth and an increase in the expression of vascular endothelial growth factor (VEGF) and neovascularization[56]. Regulation of VEGF by NO• has been confirmed by other investigators[57]. Furthermore, wild-type p53-induced transrepression of iNOS, as shown in both *in vitro* and *in vivo* conditions[58,59], provides a protective mechanism against prolonged exposure to pathological concentrations of NO•. These studies indicate that exposure of cells to a high level of NO• and its derivatives during chronic inflammationin the absence of wild-type p53 — and therefore the negative iNOS regulation — might increase the susceptibility to cancer.

There is also evidence of an association between NO• and its derivatives, and p53 mutations[60,61]. Both an increase in p53 mutations at codons 247 and 248, and an increase in iNOS expression, are found in inflamed lesions of the colon of patients with ulcerative colitis (UC)[61]. Similarly, a high expression of iNOS along with G:C to T:A transversions at codon 249 of p53 is reported in the tissue from patients with haemochromatosis[62]. In colon tumours, iNOS activity is highest in adenomas, then decreases with advancing tumour stage and is lowest in metastatic tumours[60,63]. One explanation for this reduction in iNOS is the killing of tumour-associated mononuclear cells, which produce iNOS, by FAS LIGANDS in advanced tumours[64]. Interestingly, in colon tumours there is a positive correlation between iNOS activity and G:C to A:T mutations at 5-methylcytosine sites in p53, but the rates of all other mutations vary inversely with iNOS activity[60]. There is also an association between increased iNOS expression and G:C to A:T transition mutations in p53 in stomach, brain and breast cancers[65–70]. NO• and its derivatives are, therefore, capable of causing mutations in cancer-related genes, inducing clonal expansion of mutated or aberrant cells and promoting angiogenesis — NO• can therefore act as both an endogenous initiator and a promoter in human carcinogenesis.

Animal models of inflammation

Animal models have been developed to provide a better understanding of the mechanisms that are involved in chronic inflammatory-associated carcinogenesis. There are several models for many of the diseases. The first studies — published in the 1940s — described an increased cancer incidence in areas of the body undergoing chemical or physical irritation[71,72]. Since then, there have been many animal models developed that use irritants to induce chronic inflammation. Often, if the inflammation continues over a prolonged period, cancer develops at the site of

MALONDIALDEHYDE
A naturally occurring, bioactive by-product of lipid peroxidation and prostaglandin synthesis that has the potential to damage DNA. It reacts with DNA to form adducts to deoxyguanosine and deoxyadenosine.

FAS LIGAND
A 40-kDa transmembrane protein that belongs to the tumour necrosis factor (TNF) family. It is a potent pro-apoptotic factor.

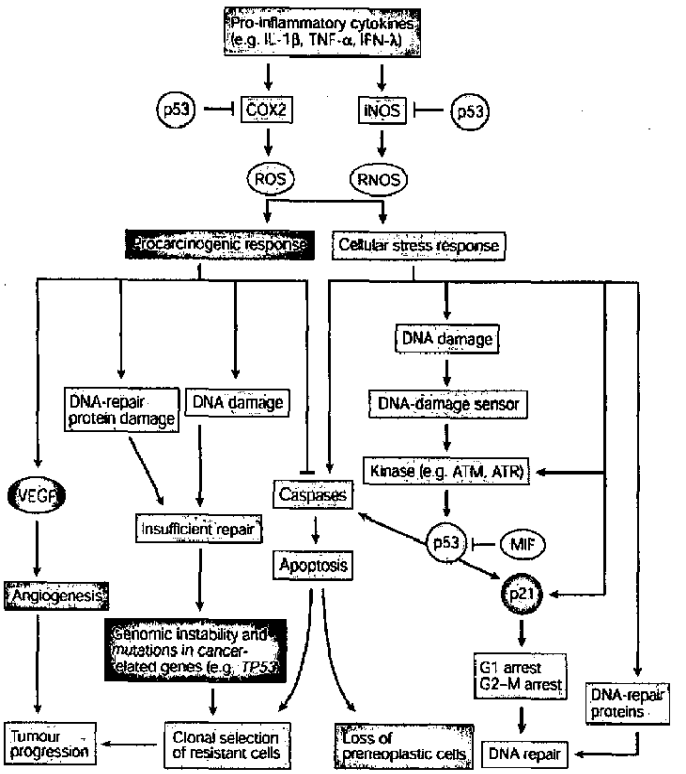

Figure 2 | **Free-radical generation, cellular stress and tumorigenesis.** Free radicals might inhibit or exacerbate tumorigenesis, depending on concentrations, the cell type, genetic background and the microenvironment in which free radicals act. Pro-inflammatory cytokines induce inducible nitric oxide synthase (iNOS) and COX2 that generate free radicals (for example, reactive oxygen species (ROS) and NO•). Both iNOS and COX2 can be transcriptionally repressed by p53 (REFS 56,152). The cellular stress response involves identification of the DNA damage, DNA repair and loss of preneoplastic cells. By contrast, insufficient repair of DNA damage causes a procarcinogenic response, triggering selection of cancerous cells and angiogenesis. ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and RAD3 related; MIF, macrophage inhibitory factor; VEGF, vascular endothelial growth factor.

irritation and inflammation. One example of such a model is mice that have been fed dextran sodium sulphate (DSS), which leads to the onset of inflammatory bowel disease and, if continued, colon cancer[73,74]. In another model, surgically opening the duodenum to the gastroesophageal junction — oesophagogastroduodenal anastomosis — which introduces mixed reflux of gastric and duodenal contents in rats, causes the onset of Barrett's oesophagus and, if continued, oesophageal cancer[75,76]. In addition, transgenic mice overexpressing the hepatitis B virus large envelope polypeptide develop inflammation and regenerative hyperplasia, and, if continued, liver cancer[77]. *Helicobacter pylori* infection in mice induces chronic inflammation and gastric atrophy, and, if continued, gastric adenocarcinoma[78].

APC GENE
The adenomatous polyposis coli gene is a tumour suppressor. Mutations in the gene are responsible for familial adenomatous polyposis and most sporadic colorectal cancers. The best understood function of APC is the destabilization of β-catenin, a key effector of the WNT signalling pathway.

Knockout models targeting key molecules involved in inflammation and inflammatory-mediated carcinogenesis also provide a better understanding of potential targets for chemoprevention.

Il-10-knockout mice. Interleukin-10 (IL-10) is an anti-inflammatory cytokine and a pleiotropic molecule with diverse effects, including the regulation of T-cell responses, the regulation of acute inflammatory responses and the regulation of free-radical release (reviewed in REF. 79). Knocking out the gene for Il-10 in mice leads to enterocolitis[80], which is similar to human inflammatory bowel disease. This is, therefore, a useful model to study UC and Crohn's disease associated with carcinogenesis. *Il-10*$^{-/-}$ mice have progressive inflammatory changes in the colon and a high incidence of colo-rectal adenocarcinomas[81]. The demonstration that the enterocolitis pathology in *Il-10*$^{-/-}$ mice can be partially improved by exogenous IL-10 administration provides additional evidence that this molecule has an important role in inflammation[81]. However, the story is complicated by an interesting observation — *Helicobacter hepaticus* has also been shown to be involved in the development of enterocolitis in *Il-10*$^{-/-}$ mice[82].

iNOS-knockout mice. iNOS can produce micromolar quantities of NO•, and is a key molecule involved in inflammatory-mediated carcinogenesis[52]. *iNOS*$^{-/-}$ mice were first generated in 1995 to test the hypothesis that iNOS defends the host against infectious agents and tumour cells at the risk of contributing to tissue damage and shock[83]. Since then, these mice have proved useful in investigating the role of iNOS in many other pathologies, including inflammation, liver regeneration, tumorigenesis and sepsis-induced hypotension (reviewed in REF. 84).

Studies have shown that *iNOS*$^{-/-}$ mice treated with the irritant trinitrobenzene develop early-phase inflammation compared with wild-type mice[85]. Others have found that genetic ablation of the *iNOS* gene confers significant resistance to trinitrobenzene-induced lethality and colonic damage, and reduces the post-translational modification of proteins, such as nitrotyrosine formation and the production of lipid peroxidation byproducts, such as malondialdehyde[86]. Also, if the *iNOS*$^{-/-}$ mice are fed DSS they show reduced signs and symptoms of colitis compared with wild-type mice, indicating that iNOS might have a crucial role in the pathology of colitis[74]. The implications of reduced inflammation of the colon in these mice remains to be determined. An interesting study by Konopka *et al.*[87] indicated that *iNOS*$^{-/-}$ mice inoculated with B16-F1 melanoma cells develop less tumours, which are associated with lower VEGF expression, than those in wild-type mice, further indicating a role for iNOS in tumour progression[56]. Another recent study found that when mice with a germline mutation in the *Apc* tumour-suppressor gene (Min mice) were fed with DSS, they had significantly accelerated colitis, dysplasia and cancer development compared with wild-type mice, indicating

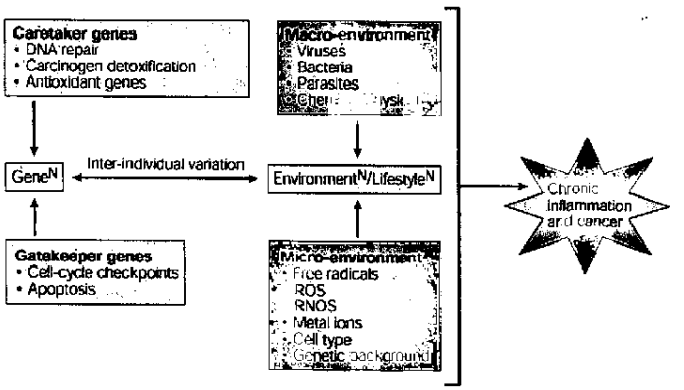

Figure 3 | **Gene–microenvironment interactions in chronic inflammation.** Genes and environmental exposures contribute to the carcinogenic process in chronic inflammatory diseases. The effects can be additive or multiplicative, and are modifiable by inter-individual variation in genetic function. We propose including antioxidant and base-excision DNA-repair genes as caretaker genes involved in maintaining genomic integrity. N, number; ROS, reactive oxygen species; RNOS, reactive nitrogen oxide species.

that this mutation is important in inflammatory-bowel-disease-associated cancer[73]. Finally, Ahn and Ohshima have shown a significant reduction in adenomas in Min/*iNOS*$^{-/-}$ mice compared with Min mice alone[88], indicating that NO$^{\bullet}$ and its derivatives have an important role in promoting colon carcinogenesis in a background of *APC* mutations.

Cox1- and Cox2-knockout mice. Cyclooxygenases catalyse the transformation of arachidonic acid into prostaglandin H2, as the first step in the biosynthesis of prostaglandins and their associated compounds. Following the discovery of two isoforms of cyclooxygenase (COX1 and COX2), many studies focused on the latter, inducible isoform (COX2) as a crucial molecule in inflammatory-mediated carcinogenesis[89]. Recent studies, using *Cox1*$^{-/-}$ and *Cox2*$^{-/-}$ mice, indicate that both of these enzymes are important in cancer development[90–94]. Recently, a third member of the cyclooxygenase family, COX3, has been cloned and characterized[95]. COX3 is selectively inhibited by pyretic drugs that alleviate pain or fever, such as acetaminophen, phencetin, antipyrine and dipyrone, and is potently inhibited by some NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs). Glycosylation is required for its activity, it is a membrane-bound 65-kDa protein (5.2 kb mRNA), and it is found mostly in the cerebral cortex and heart. The future development of *Cox3*-knockout mice will provide insight into its significance to tumorigenesis.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs). This heterogeneous group of drugs primarily target the cyclooxygenase enzymes to treat pain, fever and inflammation.

Human oxyradical overload diseases

We have already outlined some of the more prevalent oxyradical overload diseases above and in TABLE 1. In this section, we will focus on selected diseases as models to better understand the association between chronic inflammation and cancer in human tissues.

Haemochromatosis. The molecular changes that are associated with hepatocellular carcinoma (HCC) and other diseases that cause chronic liver injury, inflammation, hepatocellular necrosis and liver regeneration diseases, have been well characterized[96]. Populations that are at high risk of developing HCC are useful models for examining the association between chronic inflammation and cancer. Included in this group are chronic hepatitis B or C carriers and patients with a genetic susceptibility to HCC, such as those with haemochromatosis. If the process of chronic liver injury and inflammation continues for many years, liver cirrhosis occurs, which then markedly increases the risk of developing HCC. These findings indicate that the modulation of cell proliferation and/or inflammatory responses that is associated with necroinflammatory diseases is responsible for the increased probability of neoplastic transformation of HCC precursor cells.

Haemochromatosis is an inherited cancer-prone disease that is characterized by excess build-up of iron in the liver, leading to tissue damage by means of oxyradical overload[97]. Genes involved in hereditary haemochromatosis include *HFE*, a gene on chromosome 1q, and genes encoding the transferrin receptor 2, ferroportin and ferritin H[98]. The resulting excessive accumulation of iron in hepatocytes causes hepatocellular injury, leading to fibrosis and cirrhosis (reviewed in REF. 99). The metal ions and the free radicals that are produced can induce a number of alterations in cellular macro-molecular targets, as well as point mutations in genes[49,100–102]. Increased cell division contributes to the conversion of DNA lesions into point mutations. We have tested the hypothesis that an increase in the *TP53* mutation load in the liver of haemochromatosis patients can arise before cancer development through several different mutagenic pathways[103]. Reactive aldehydes — such as 4-hydroxynonenal, malondialdehyde and crotonaldehyde — that are produced during lipid peroxidation, can generate exocyclic DNA adducts (addition of a 5–6-membered exocyclic ring on DNA bases) and point mutations[104–106]. We have reported previously that 4-hydroxynonenal preferentially induced G:C to T:A transversions at p53 codon 249 in TK6 lymphoblastoid cells[62]. There is also evidence of increased lipid peroxidation and etheno-DNA adducts (addition of a 5-membered exocyclic ring on DNA bases) in haemochromatosis patients[107–109].

Ulcerative colitis. UC is a chronic inflammatory disease of unknown aetiology, which is associated with an increased risk of developing colon cancer. The colonic mucosa in patients with UC shows uniform and continuous inflammation with ulceration and micro-abscess formation. Neutrophilic infiltration and inflammatory reactions have been linked with the formation of these lesions. The precursor neoplasm of UC differs in histology and morphology to that of sporadic colon cancer. Whereas adenomatous polyps are considered to be the main precursor of the sporadic disease, UC-associated cancers involve the development of epithelial dysplasia[110]. Several genetic and epigenetic changes have been described in UC that might

BIOMARKER
A specific biochemical that provides a measure of a biological response to an endogenous or exogenous agent. The biological responses might be at the molecular, cellular or whole-organism level.

Table 2 | **Key genetic polymorphisms for genes involved in DNA repair and antioxidation**

	Polymorphism	Normal function	Cancer site
DNA repair genes*			
OGG1	S326C	Repairs 8-oxo-dG and other altered DNA bases	Oesophagus, lung, prostate
XRCC1	R194W	Repairs DNA-base damage and single-strand breaks	Bladder, breast, lung, SCCHN, stomach
BRCA2	N372H	Multiple, including DNA-damage response, DNA repair, chromatin remodelling, cell-cycle control	Breast
Antioxidant genes			
MnSOD	Val to Ala change in the -9 position	Converts $O_2^{\bullet-}$ to H_2O_2	Breast
GPx	Pro198Leu	Converts H_2O_2 to H_2O	Lung

*Information for DNA-repair genes was obtained from REF. 130. GPx, glutathione peroxidase; MnSOD, manganese superoxide dismutase; SCCHN, squamous-cell carcinoma of the head and neck.

be responsible for colon carcinogenesis[111], and these differ in frequency and timing compared with sporadic cancer[112–115]. For instance, alterations in p53 — a late event in the molecular pathogenesis of sporadic colorectal cancer — occur earlier in the process of UC-associated carcinogenesis[61,116]. As described above, we have identified UC patients with a high p53 mutation load and iNOS activity in areas of active inflammation[61]. This correlation indicates an association between high levels of free radicals and p53 mutations in the inflamed part of colon, thereby increasing the risk of developing colon cancer.

Viral hepatitis. Some 40% of all HCCs worldwide occur in hepatitis B virus (HBV)-infected individuals[96]. HBV has been studied extensively, and might have several roles in HCC initiation. Many early studies focused on viral integration, because most HCCs in HBV carriers contain HBV DNA sequences apparently randomly integrated into the host chromosomal DNA[117]. This integration and subsequent chromosomal alterations might result in the loss of tumour-suppressor genes that are necessary for cell-cycle control, differentiation and apoptosis.

Another effect of viral infection receiving increasing attention is hepatocellular necrosis, inflammation and liver regeneration. The impact of viral hepatitis, characterized by liver-cell injury or liver oedema induced by infiltration of inflammatory cells[118], can lead to DNA damage and the modification of key proteins that are involved in carcinogenesis, which is significant in the genesis of HCC. This was shown in a transgenic mouse model of HBV, in which sustained accumulation of the DNA adduct 8-oxo-2′-deoxyguanosine (8-oxo-dG) starts early in life and increases progressively with advancing disease, leading to HCC[119]. 8-oxo-dG — one of the main DNA adducts that is formed by oxidative stress — can produce a number of missense mutations in cancer-related genes.

Many studies have focused on the influence of the hepatitis B virus x (HBx) protein on HBV-mediated hepatocarcinogenesis. HBx binds to many cellular proteins and also acts as a co-transcription factor to regulate many cellular and viral genes. The HBx and inflammatory components of hepatocarcinogenesis are probably not mutually exclusive. The HBx protein has been shown to interact with inflammation-associated molecules and activate pro-inflammatory cytokines[120–125]. Also, both inflammatory species and HBx can deregulate cell-cycle checkpoint controls, block p53-mediated apoptosis and inhibit DNA repair, and therefore contribute to the selection of cells that are genetically unstable, some of which accumulate as unrepaired oncogenic mutants.

Although hepatitis C virus (HCV) is a significant cause of disease-associated morbidity and mortality worldwide, its role as an aetiological agent in HCC was not recognized until 1989 (REFS 126,127). Much of our knowledge about its influence on HCC has since come from large epidemiological studies. The mechanisms involved, however, are not clearly understood. Due to the impact of HCV on viral hepatitis and associated inflammation, HCV, in combination with mechanisms involved in chronic inflammation, can have potent effects on liver carcinogenesis.

Table 3 | **Human chemoprevention trials in oxyradical overload diseases**

Disease	Cancer inhibited/ potentially inhibited	Chemopreventive agent	References
Prospective, randomized control trials			
HBV, HCV	HCC	Interferon-α	135,137–139
HBV	HCC	Sho-saiko-to (*TJ-9*)	161
Helicobacter pylori	Gastric	Ascorbic acid, β-carotene, α-tocopherol	162,163
HPV	Cervical	All-*trans* retinoic acid, β-interferon	164,165
Retrospective studies			
Crohn's disease	Colon	NSAIDs	166
UC	Colon	5-ASA, sulphalazine, mesalazine, ursudiol, folates	167–171
HBV, HCV	HCC	Neominophagen C, glycyrrhizin, selenium, vitamins A and E, β-carotene	172–176
HPV	Cervix	Folates, carotenoids, vitamins C and E	177
Helicobacter pylori	Gastric	NSAIDs, aspirin	178

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HPV, human papillomavirus; NSAIDs, non-steroidal anti-inflammatory drugs; UC, ulcerative colitis.

Gene–microenvironment interactions

Molecular epidemiology of human cancer risk is the study of inter-individual variation and gene–environment interactions through a multidisciplinary effort — including epidemiology, molecular genetics, cell biology, biochemistry, statistics and bioethics. Recognizing that cancer is a genetic disease involving multiple genes, molecular epidemiology uses BIOMARKERS of cancer risk that will elucidate multiple gene–environment interactions, in which several genes and exposures work interactively (FIG. 3).

Any inherited variation in the genetic code that alters protein expression, function or localization can increase the susceptibility to cancer. Genetic polymorphisms — changes in the nucleotide sequence that are present in at least 1% of the population — are an important example. The presence of genetic polymorphisms in genes controlling important cellular functions, such as DNA repair, apoptosis and the cell cycle, might affect cancer risk in an individual. The concentration and activity of DNA-repair enzymes are altered in inflammation[128,129], and cells protect themselves from the damage caused by free radicals through DNA repair. It is therefore important to assess genetic polymorphisms in DNA-repair genes in patients with chronic inflammation. Recent studies[130] have shown an association between polymorphisms in DNA-repair genes and a variety of human cancers (TABLE 2). Generation of free radicals through endogenous cellular processes creates an oxidative environment which, if not removed by the delicately balanced antioxidant enzyme machinery, causes oxidative damage in DNA and proteins. Polymorphism of the gene encoding the antioxidant enzyme manganese superoxide dismutase (MnSOD), which converts $O_2^{\bullet-}$ to H_2O_2, alters protein trafficking and is associated with increased breast cancer risk[131]. Furthermore, a polymorphism at Pro198Leu in the glutathione peroxidase 1 gene — which converts H_2O_2 to water — is associated with an increased risk of lung cancer[132]. It would be interesting to extend these studies to investigate cancer-prone, oxyradical overload diseases.

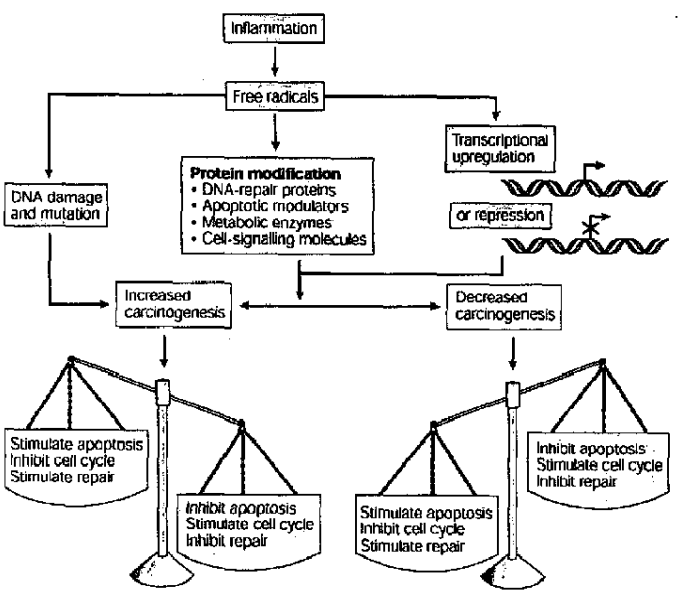

Figure 4 | **Chronic inflammation and production of free radicals regulate multiple cellular processes.** Free radicals, released at the site of inflammation, not only damage DNA, but also modify cancer-related proteins and regulate transcription. Often, if the insult continues over a prolonged period of time, the scales are tipped in favour of increased tumorigenesis, with DNA damage and mutation, an inhibition of apoptosis, a stimulation of the cell cycle/proliferation and an inhibition of DNA repair.

Treatment and protection strategies

Targeting the genes that are involved in chronic inflammation with an increased cancer risk might provide an effective therapy. Current targets for gene therapy in acute inflammatory diseases have been reviewed elsewhere[1,3,133]. They include targeting molecules that suppress inflammation (for example, tumour necrosis factor-α and -β, and interleukin-1, 4, 10 and 13), block protease activity (α1-antitrypsin, tissue inhibitors of metalloproteinases), reduce free radical and superoxide production (catalase, superoxide dismutase and glutathione peroxidase), inhibit apoptosis of lymphoid-cell and somatic-cell populations (BCL2, BCL-X_L and iNOS) or promote wound healing (such as growth factors).

Prevention of exposure or eradicating the exposure soon after it occurs is the most logical strategy for reducing the risk of cancer in oxyradical overload diseases with an environmental component. Changing behaviour (such as stopping drug addicts sharing needles and increasing the transmission of hepatitis viruses), vaccinations (for HBV and human papillomavirus) and early treatment (such as praziquantel for *Schistosomiasis* infection[73,134] or interferon for hepatitis[135–139]) can also be effective. Surveillance, screening and prophylactic surgery in non-vital organs — such as the colon of UC patients — can also be useful. All approaches are complimented by chemoprevention strategies.

Chemoprevention targets pathways that are involved in the synthesis and quenching of cancer-causing molecules. In chronic inflammation, inhibiting free-radical synthesis or scavenging free radicals are attractive approaches. This can be done by dietary modulation, micronutrient supplementation and naturally or synthetically derived drugs. TABLE 3 lists some key prospective and retrospective chemoprevention trials that point towards the usefulness of this strategy to prevent cancer in patients with oxyradical overload diseases.

Conclusions and perspectives

The term 'oxyradical overload' encompasses a wide range of diseases that are associated with an increased cancer risk. Key reasons for this increased risk include DNA damage, protein modification and changes in the transcriptional activation and/or repression of genes that are responsible for cellular homeostasis. Any chronic shift in the maintenance of this cellular homeostasis can lead to permanent changes associated with carcinogenesis (FIG. 4).

Because of the complexity of free radicals, it has been difficult to delineate the specific role of each radical in carcinogenesis. We argue that NO• and its derivatives are not only key molecules that damage DNA, but they also modify the structure and function of proteins that are involved in the maintenance of cellular integrity and that promote angiogenesis. However, there are 'two faces' of NO•, and further study is required before iNOS and NO• should be considered as targets for the prevention of chronic-inflammatory-induced carcinogenesis in humans. At present, evidence favours the use of COX2 inhibitors as chemoprevention agents[89]. Combination strategies using both iNOS and COX2 inhibitors, or using single agents that target both iNOS and COX2 (for example, dexamethasone) using NO•-releasing NSAIDs, or targeting the NF-κB pathway might prove to be useful approaches for reducing cancer that is associated with chronic inflammation[140–144]. As a cautionary note, in some cases, targeting iNOS or COX2 has resulted in an exacerbation of carcinogenesis in animal models (reviewed in REFS 52,89).

Careful attention by means of chemopreventive or other regimens must be given to patients with oxyradical overload diseases to stop, or even reverse, carcinogenesis before the cancer becomes clinically observable. Towards this end, to our knowledge, there have been no retrospective or prospective chemoprevention studies published for many oxyradical overload diseases other than those listed in TABLE 3. There is a need for more long-term, prospective, randomized studies to provide insight into the agents that can protect the many people with oxyradical overload diseases from cancer development. Although the best way to test the efficacy of chemopreventive agents is by determining their ability to reduce cancer incidence, this is rarely done in a prospective manner because of the expense, time and manpower required. This is why in high cancer risk, oxyradical overload diseases, cancer as an end point is rarely used to test the efficacy of chemopreventive agents. Instead, intermediate biomarkers can be useful as predictors of outcome. However, few markers have been properly evaluated to directly link them to cancer outcome. The impact of free radicals on DNA is thought to be the main link between free radicals and cancer formation, and as p53 is a key molecule involved in carcinogenesis, quantification of *TP53* mutation load is a promising intermediate-effect marker to predict cancer risk or to test the efficacy of chemopreventive agents. Measuring the *TP53* mutation load or the frequency of mutated alleles in non-tumorous tissue might indicate previous carcinogen exposure and identify individuals at increased cancer risk. As only an extremely small number of cells harbour a particular mutation at the early stage of a disease, the development of a highly sensitive genotypic assay has been important in allowing the detection of low-frequency mutations in normal-appearing human tissues, as well as in cells that are exposed to an environmental carcinogen[145–147]. The analysis of cancer-related gene fragments in serum or other bodily specimens, such as stools, might also provide useful intermediate biomarkers[148,149].

Because free radicals directly modify DNA and proteins, the measurement of damage products is useful for assessing risk or for developing chemoprevention strategies in oxyradical overload diseases. These markers, as well as measuring p53 post-translational modification and accumulation[23], assess overall exposure of the body to inflammatory stress. Some promising specific markers used in the monitoring of nitrosative stress and chemoprevention of this stress include *N*-nitrosoproline, *N*-nitrosamino acid and NO_3^- in urine or plasma; 8-oxo-dG, 8-nitrosoguanosine, exocyclic etheno- and malondialdehyde–DNA adducts in leukocytes or target tissue; and 3-nitrotyrosine protein adducts[12,52,150,151]. So far, the consequences of these effect markers on human carcinogenesis are unknown and need to be evaluated so that useful chemopreventive strategies can be properly assessed.

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Online links

DATABASES

The following terms in this article are linked online to:

Cancer.gov: http://www.cancer.gov/cancer_information/
brain cancer | breast cancer | colon cancer | hepatocellular carcinoma | lung cancer | oesophageal cancer | stomach cancer

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
APC | *Cox1* | COX1 | *Cox2* | COX2 | ferritin H | ferroportin | FOS | glutathione peroxidase 1 | *HFE* | Il-10 | IL-10 | iNOS | JUN | MnSOD | MYC | p53 | *transferrin receptor 2* | VEGF | WAF1

OMIM: http://www.ncbi.nlm.nih.gov/Omim/
Crohn's disease | haemochromatosis | ulcerative colitis

FURTHER INFORMATION

For further information on Barrett's oesophagus:
http://pathology2.jhu.edu/beweb/
For further information on chemoprevention:
http://www.mdanderson.org/topics/chemoprev/
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